

## CLAIMS

1. A method for immobilizing biomolecules, which method comprises contacting a solution containing a biomolecule or biomolecules provided with at least one tag with an immobilization substrate which has (i) binding sites for the biomolecule tag or tags, and (ii) activated reactive groups which are capable of forming covalent bonds with the biomolecule or biomolecules.
2. The method according to claim 1, comprising the steps of:
  - a first step wherein the reactive groups of the immobilization substrate which are capable of forming a covalent bond with the biomolecule or biomolecules to be immobilized are activated;
  - a second step wherein a solution containing the biomolecule or biomolecules to be immobilized is reacted with the immobilization substrate following the first step, and wherein, in the second step, the biomolecule or biomolecules are immobilized on the immobilization substrate through interaction between the tag or tags and tag-binding sites of the immobilization substrate and covalent bonds formed between the reactive groups and the biomolecule or biomolecules.
3. The method according to claim 2, wherein the reactive groups are carboxyl groups, and in the second step, an amine coupling is formed between the carboxyl groups and an amino group on the biomolecule to be immobilized.
4. The method according to claim 2 or 3, wherein the tag is a histidine tag, and in the second step, an interaction is effected between the histidine tag and the immobilization substrate.
5. The method according to claim 4, wherein, in the second step, an interaction is effected between the histidine tag and the immobilization substrate through a complex.
6. The method according to claim 5, wherein, in the second step, an interaction is effected between the histidine tag and the immobilization substrate through a metal ion chelate.

7. The method according to claim 6, wherein, in the second step, an interaction is effected between the histidine tag and the immobilization substrate through  $\text{Ni}^{2+}$ -nitrilotriacetic acid (Ni-NTA).

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8. The method according to claim 6, wherein, in the second step, an interaction is effected between the histidine tag and the immobilization substrate through  $\text{Ni}^{2+}$ -iminodiacetic acid (Ni-IDA).

10 9. The method according to any one of claims 1 to 3, wherein the tag-binding site of the immobilization substrate is an antibody to the tag.

10. The method according to claim 9, wherein the tag is a histidine tag, the antibody is an anti-histidine antibody and, in the second step, an interaction is effected between  
15 the histidine tag and the immobilization substrate through the anti-histidine antibody.

11. The method according to any one of claims 1 to 3, wherein the tag is an inherent part of a native biomolecule.

20 12. The method according to any one of claims 1 to 11, wherein the biomolecule is a protein.

13. A method for determining biomolecule-low molecular weight compound affinity and/or kinetics comprising:

25 a step for reacting a sample containing a low molecular weight compound or compounds to be determined with an immobilization substrate to which a biomolecule or biomolecules have been immobilized using the method for immobilizing biomolecules as defined in any one of claims 1-12, and

a step for determining the affinity and/or kinetics of the low molecular weight  
30 compound or compounds contained in the sample for the biomolecule or biomolecules immobilized on the immobilization substrate.

14. The method according to claim 13, wherein the affinity and/or kinetics of a biomolecule and a low molecular weight compound is determined using the principle of surface plasmon resonance (SPR) in the step for determining affinity and/or kinetics.
- 5 15. The method according to claim 13 or 14, wherein the biomolecule is a protein.
16. A method for determining protein-protein affinity and/or kinetics comprising:  
a step for reacting a sample containing a protein or proteins to be determined  
with an immobilization substrate which has a protein or proteins immobilized thereon  
10 using the method for immobilizing biomolecules as defined in any one of claims 1 to 12, and  
a step for determining the affinity and/or kinetics of the protein or proteins contained in the sample for the protein or proteins immobilized on the immobilization substrate.
- 15 17. The method according to claim 16, wherein the affinity and/or kinetics of a protein in the sample for an immobilized protein is determined using the principle of surface plasmon resonance (SPR) in the step for determining affinity and/or kinetics.
- 20 18. An immobilization substrate comprising at least one immobilized biomolecule, wherein the biomolecule or biomolecules have been immobilized by the method defined in any one of claims 1 to 12.
19. The immobilization substrate of claim 18, which comprises;  
25 a substrate, and  
polysaccharide chains arranged on the substrate, into which are introduced reactive groups capable of forming covalent bonds with a biomolecule or biomolecules to be immobilized thereon,  
wherein the biomolecule or biomolecules interact with the polysaccharide chain  
30 through a chelate and form covalent bonds with the reactive groups.
20. The method according to claim 18 or 19, wherein the biomolecule is a protein.